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FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
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                                               ENTRY     SESSION
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INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
      AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS,
      BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB,
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=> s myocardin (w) (polypeptide or protein)
          2   FILE BIOTECHABS
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  15 FILES SEARCHED...
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  27 FILES SEARCHED...
          1   FILE IFIPAT
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=> file hits -dgene
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                                               ENTRY     SESSION
FULL ESTIMATED COST                             1.71        1.92

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=> s l1
L2 8 L1

=> dup rem l2
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L3 4 DUP REM L2 (4 DUPLICATES REMOVED)

=> d l3 bib ab 1-4

L3 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:253441 CAPLUS
DN 139:114987
TI Myocardin is a critical serum response factor cofactor in the transcriptional program regulating smooth muscle cell differentiation
AU Du, Kevin L.; Ip, Hon S.; Li, Jian; Chen, Mary; Dandre, Frederic; Yu, William; Lu, Min Min; Owens, Gary K.; Parmacek, Michael S.
CS Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104, USA
SO Molecular and Cellular Biology (2003), 23(7), 2425-2437
CODEN: MCEBD4; ISSN: 0270-7306
PB American Society for Microbiology
DT Journal
LA English
AB The SAP family transcription factor myocardin functionally synergizes with serum response factor (SRF) and plays an important role in cardiac development. To det. the function of myocardin in the smooth muscle cell (SMC) lineage, we mapped the pattern of myocardin gene expression and examd. the mol. mechanisms underlying transcriptional activity of myocardin in SMCs and embryonic stem (ES) cells. The human and murine myocardin genes were expressed in vascular and visceral SMCs at levels equiv. to or exceeding those obsd. in the heart. During embryonic development, the myocardin gene was expressed abundantly in a precise, developmentally regulated pattern in SMCs. Forced expression of myocardin transactivated multiple SMC-specific transcriptional regulatory elements in non-SMCs. By contrast, myocardin-induced transactivation was not obsd. in SRF-/- ES cells but could be rescued by forced expression of SRF or the SRF DNA-binding domain. Furthermore, expression of a dominant-neg. myocardin mutant protein or small-interfering-RNA-induced myocardin knockdown significantly reduced SM22.alpha. promoter activity in SMCs. Most importantly, forced expression of myocardin activated expression of the SM22.alpha., smooth muscle .alpha.-actin, and calponin-h1 genes in undifferentiated mouse ES cells. Taken together, these data demonstrate that myocardin plays an important role in the SRF-dependent transcriptional program that regulates SMC development and differentiation.
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
DUPLICATE 1
AN 2003-05351 BIOTECHDS
TI New myocardin polypeptides and polynucleotides, useful for respecifying non-cardiac cells, stimulating cardiac tissue regeneration, and for treating cardiovascular disorders, such as myocardial infarction and hypertension;
retro virus, adeno virus, adeno-associated virus, vaccina virus, herpes virus, polyoma virus or Sindbis virus vector-mediated gene transfer and expression in fibroblast cell for use in gene therapy
AU OLSON E N; WANG D
PA UNIV TEXAS SYSTEM
PI WO 2002060946 8 Aug 2002
AI WO 2001-US50606 21 Dec 2001
PRAI US 2000-257716 21 Dec 2000; US 2000-257716 21 Dec 2000
DT Patent
LA English
OS WPI: 2002-732693 [79]
AB DERWENT ABSTRACT:
NOVELTY - An isolated polynucleotide (I) encoding a ***myocardin*** ***polypeptide*** , is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid segment (II) comprising at least 15 contiguous nucleotides of any of the sequences of 4959, 2424, 3063 or 4960 base pairs, fully defined in the specification; (2) an expression cassette (III) comprising (I) operably linked to a regulatory sequence; (3) a transformed host cell (IV) comprising (I), and a promoter heterologous to the polypeptide coding region, where the promoter directs expression of the ***myocardin*** ***polypeptide*** ; (4) using (M1) a host cell comprising an expression cassette with (I), and a promoter active in the host cell; (5) a fusion protein (V) comprising a ***myocardin*** ***protein*** or peptide fused to a second protein or peptide; (6) modulating (M2) the phenotype of a non-cardiomyocyte cell to include one or more phenotypic functions of a cardiomyocyte cell; (7) generating (M3) a cardiomyocyte comprising introducing into a cardiac fibroblast (III) and a promoter active in the fibroblast, where the promoter directs the expression of the polypeptide; (8) stimulating (M4) cardiac tissue regeneration comprising inhibiting the function of myocardin in a post-mitotic cardiomyocyte; (9) expressing (M5) a ***myocardin*** ***polypeptide*** in a host cell; (10) a monoclonal
antibody (VI) that binds immunologically to a polypeptide comprising any of fully defined sequences of 807, 938 or 935 amino acids, given in the specification; (11) a polyclonal antisera (VII), antibodies of which binds immunologically to a polypeptide comprising any of fully defined sequences of 807, 938 or 935 amino acids, given in the specification; (12) a hybridoma cell (VIII) that produces a monoclonal antibody that binds immunologically to a polypeptide comprising any of fully defined sequences of 807, 938 or 935 amino acids, given in the specification; (13) a non-human transgenic animal (IX) comprising an expression cassette; (14) screening (M6) for a modulator of myocardin expression; (15) screening (M7) a candidate substance for myocardin binding activity; (16) a peptide (X) of 8-50 residues comprising at least 8 consecutive residues of any of fully defined sequences of 807, 938 or 935 amino acids, given in the specification; (17) a non-human transgenic animal (XI) comprising a defective germ-line myocardin allele; (18) treating (M8) a heart disease, including cardiomyopathy comprising administering to an animal suffering from an expression cassette comprising a polynucleotide encoding a myocardin peptide or protein and a promoter operable in eukaryotic cells; (19) treating (M9) a heart disease, including cardiomyopathy comprising the step of providing to an animal suffering from a myocardin antisense nucleic acid; (20) decreasing (M10) mortality in a subject with heart failure comprising inhibiting the function of myocardin in post-mitotic cardiomyocytes in the subject; (21) decreasing (M11) mortality in a subject with heart failure comprising increasing the level of myocardin in fibroblasts to generate

cardiomyocytes in the subject; (22) decreasing (M12) morbidity in a subject with heart failure comprising inhibiting the function of myocardin in post-mitotic cardiomyocytes in the subject; (23) decreasing (M13) morbidity in a subject with heart failure comprising increasing the level of myocardin in fibroblasts to generate cardiomyocytes in the subject; and (24) screening (M14) for a candidate substance for an effect on myocardin regulation of cardiomyocyte development.

BIOTECHNOLOGY - Preferred Polynucleotide: The ***myocardin*** ***polypeptide*** encoded by (I) comprises any of fully defined sequences of 807, 938 or 935 amino acids, given in the specification. The polynucleotide sequence comprises any of the sequences of 4959, 2424, 3063 or 4960 base pairs, fully defined in the specification. The polynucleotide further comprises a promoter operable in eukaryotic cells. Preferred Nucleic Acid: The segment of the (II) is 15, 20, 25, 30, 35, 50, 100, 150, 250, 500, 1000 or 2000 nucleotides in length. The number of contiguous nucleotides is 20, 25, 30, 35 or 50. Preferred Expression Cassette: (III) encodes a polypeptide with any of fully defined sequences of 807, 938 or 935 amino acids, given in the specification. The polynucleotide sequence comprises any of the sequences of 4959, 2424, 3063 or 4960 base pairs, fully defined in the specification. The regulatory sequence comprises a promoter heterologous to the coding sequence, where the promoter is a tissue specific promoter, preferably a muscle specific promoter. The muscle specific promoter is myosin light chain-2 promoter, alpha actin promoter, troponin 1 promoter, Na+/Ca2+ exchanger promoter, dystrophin promoter, creatine kinase promoter, alpha7 integrin promoter, brain natriuretic peptide promoter, alpha B-crystallin/small heat shock protein promoter, alpha myosin heavy chain promoter or atrial natriuretic factor promoter. The muscle specific promoter is a cardiac muscle specific promoter, preferably an alpha-myosin heavy chain or ANF. The promoter is alternatively an inducible or a constitutive promoter. The expression cassette is contained in a gene delivery vector, which is a viral vector. The viral vector is a retroviral vector, an adenoviral vector, an adeno-associated viral vector, a vaccina viral vector, a herpesviral vector, a polyoma viral construct or a Sindbis viral vector. The expression cassette further comprises a polyadenylation signal, and a second polynucleotide encoding a second polypeptide. The second polypeptide is a cardiac transcription factor. Preferred Host Cell: The host cell is further defined as a prokaryotic or eukaryotic host cell. Preferred Peptide: The peptide further comprises 10 or 12 consecutive residues of any of fully defined sequences of 807, 938 or 935 amino acids, given in the specification. Preferred Method: (M1) comprises culturing the host cell under conditions suitable for the expression of the ***myocardin*** ***polypeptide*** . (M2) comprises comprising introducing into the non-cardiac cell (III), where the promoter directs the expression of the polypeptide. The non-cardiomyocyte cell is a fibroblast. The method further comprises measuring cardiac lineage markers. The expression cassette further comprises a second polynucleotide encoding a second polypeptide. The second polypeptide is a cardiac transcription factor, which is GATA4. The second polynucleotide is under the control of a second promoter. The first and second polynucleotide are the under control of the same promoter. The method further comprises introducing a second expression cassette into the non-cardiomyocyte cells, where the second expression cassette comprises a polynucleotide encoding a second polypeptide and a second promoter active in the non-cardiomyocyte cells, where the second promoter directs the expression of the second polypeptide. The measuring comprises RNA hybridization, PCR, RT-PCR or Western analysis. The expression vector in (M3) comprises a lipid based vector or a viral vector. The viral vector is a retroviral vector, an adenoviral vector, an adeno-associated viral vector, a vaccina viral vector, a herpesviral vector, a polyoma viral construct or a Sindbis viral vector. The promoter is heterologous to the coding sequence. The promoter is a tissue specific promoter or a muscle specific promoter. The muscle specific promoter is a cardiac muscle specific promoter. The expression cassette further comprises a second polynucleotide encoding a second polypeptide, where the second polypeptide is a cardiac transcription factor. The cardiac transcription factor is GATA4. The second polynucleotide is under the control of a second promoter active in a cardiac fibroblast. The first and second polynucleotide are under the control of the same promoter. The method further comprises introducing into the fibroblasts a the second expression cassette comprising a polynucleotide encoding a second polypeptide and a second promoter active in the fibroblast, where the second promoter directs the expression of the second polypeptide. The expression cassette further comprises a polyadenylation site, and a selectable marker, which is an immunologic marker. The inhibiting in (M4) comprises providing to the postmitotic cardiomyocyte an antisense nucleic acid that inhibits transcription or translation of a myocardin mRNA. The providing comprises introducing into the post-mitotic cardiomyocyte an expression cassette encoding myocardin antisense RNA and a promoter active in the cardiomyocytes. (M5) comprises introducing into the host cells (III), the polynucleotide being positioned under control of a promoter operable in the host cell. The expression cassette in (IX) comprises a polynucleotide encoding a myocardin peptide or protein and a promoter operable in eukaryotic cells, the promoter being heterologous to the myocardin peptide or protein encoding region. (M6) comprises: (a) providing a cell that expresses a ***myocardin*** ***polypeptide*** ; (b) contacting the ***myocardin*** ***polypeptide*** with a candidate substance; and (c) measuring the expression of myocardin, where a difference in myocardin expression, indicates that the candidate substance is a modulator of myocardin expression. The modulator enhances or inhibits myocardin expression. The candidate modulator is a pharmaceutical composition. (M7) comprises providing a ***myocardin*** ***polypeptide*** , contacting the ***myocardin*** ***polypeptide*** with the candidate substance, and determining the binding of the candidate substance to the ***myocardin*** ***polypeptide*** . The assay is performed in a cell free system, in a cell or in vivo. The candidate substance is an inhibitor or enhancer of myocardin. The cardiomyopathy in (M8) is myocardial infarction or hypertension. The promoter is a cardiac specific promoter. The expression cassette is comprised within a replication-defective expression vector. The replication defective expression vector is a viral vector, where the viral vector is a retroviral vector, an adenoviral vector, an adeno-associated viral vector, a vaccina viral vector, a herpesviral vector, a polyoma viral construct or a Sindbis viral vector. (M14) comprises: (1) providing myocardin and GATA4 to a cell; (2) admixing myocardin and GATA4 in the presence of the candidate substance; and (3) measuring the effect of the candidate substance on the expression of a cardiac lineage marker, where a difference in the expression of the cardiac lineage marker, as compared to an untreated cell, indicates that the candidate substance effects myocardin regulation of cardiomyocyte development. The measuring comprises polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR or RNA hybridization. The measuring comprises immunologic detection of myocardin, where measuring comprises

an enzyme linked immunosorbent assay (ELISA), or immunohistochemistry. The cell is located in an animal. The cell is a fibroblast, preferably a cardiomyocyte. The cardiac lineage marker is Nkx2.5. The modulator increases or decreases the expression of the cardiac lineage marker. Preferred Transgenic Animal: The promoter in (IX) is constitutive, tissue specific or inducible. The animal is a mouse. The non-human transgenic animal of (XI) comprises two defective germ-like myocardin alleles.

ACTIVITY - Cardiant; Hypotensive. The role of myocardin in cardiomyocyte differentiation was confirmed after mRNA from a dominant negative myocardin mutant was injected into Xenopus embryos. A dramatic reduction in the expression of transcripts for cardiac alpha-actin and alpha-tropomyosin was observed. The effects on cardiac differentiation were highly specific as demonstrated by the normal overall appearance of the embryo. Also observed was a dose-dependent reduction in expression of cardiac markers, such that approximately 90% of injected embryos exhibited a reduction or complete elimination of cardiac gene expression.

MECHANISM OF ACTION - Gene therapy.

USE - The compositions and methods of the present invention are useful for respecifying non-cardiac cells, stimulating cardiac tissue regeneration, and for treating cardiovascular disorders, such as myocardial infarction and hypertension, and for screening of compounds for various abilities to interact and/or affect myocardin expression or function (claimed).

ADMINISTRATION - Routes of administration of the active compositions of the present invention include oral, intranasal, buccal, rectal, vaginal or topical, intradermal, subcutaneous, intramuscular, intraperitoneal l or intravenous. No dosages details given.

EXAMPLE - No suitable example given. (175 pages)

L3 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
DUPLICATE 2
AN 2003-11632 BIOTECHDS
TI Novel isolated polynucleotide encoding human or murine myocardin 1 polypeptide, useful for modulating phenotype of non-cardiomyocyte cell e.g., fibroblast, to include phenotypic functions of cardiomyocyte cell; vector-mediated gene transfer to host cell for heart disease therapy and gene therapy
AU OLSON E N; WANG D
PA OLSON E N; WANG D
PI US 2002164735 7 Nov 2002
AI US 2001-29217 21 Dec 2001
PRAI US 2001-29217 21 Dec 2001; US 2000-257761 21 Dec 2000
DT Patent
LA English
OS WPI: 2003-247258 [24]
AB DERWENT ABSTRACT:
NOVELTY - An isolated polynucleotide (I) encoding ***myocardin*** ***polypeptide*** , is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a myocardin peptide (II) of 8-50 residues comprising at least 8 consecutive residues of a fully defined murine myocardin 1 polypeptide sequence (S1) of 807 or 935 amino acids, or of a fully defined human myocardin 1 polypeptide sequence (S2) of 807 or 938 amino acids. All sequences are as given in the specification; (2) an isolated nucleic acid segment (III) comprising at least 15 contiguous nucleotides of a fully defined sequence (S3) of 4959, 2424, 3063 or 4960 nucleotides, as given in the specification; (3) an expression cassette (IV) comprising (I) operably linked to a regulatory sequence; (4) a transformed host cell (V) comprising (I), and a promoter heterologous to the polypeptide coding region, where the promoter directs expression of the ***myocardin*** ***polypeptide*** ; (5) a fusion protein (VI) comprising ***myocardin*** ***protein*** or (II) fused to a second protein or peptide; (6) stimulating (M1) cardiac tissue regeneration, involves inhibiting the function of myocardin in a post-mitotic cardiomyocyte; (7) preparation of ***myocardin*** ***polypeptide*** by introducing into the host cells (IV) under control of a promoter operable in the host cell; (8) a monoclonal antibody (VII) that binds immunologically to polypeptide comprising (S1) or (S2) or its antigenic fragment; (9) a polyclonal antisera, antibodies of which bind immunologically to polypeptide comprising (S1) or (S2) or its antigenic fragment; (10) a hybridoma cell that produces (VII); (11) a non-human transgenic animal (VIII) comprising an expression cassette which comprises polynucleotide encoding ***myocardin*** ***protein*** or peptide, and a promoter operable in eukaryotic cells, the promoter being heterologous to the myocardin peptide or protein encoding region; (12) a non-human transgenic animal (IX) comprising a defective germ-line myocardin allele; (13) treating (M2) a heart disease, including cardiomyopathy, involves administering to an animal suffering from the disease an expression cassette comprising polynucleotide encoding ***myocardin*** ***protein*** or peptide, and a promoter operable in eukaryotic cells; (14) treating heart disease including cardiomyopathy, providing to the animal a myocardin antisense nucleic acid; (15) decreasing mortality or morbidity in a subject with heart failure by inhibiting the function of myocardin in post-mitotic cardiomyocytes in the subject, or by increasing the level of myocardin in fibroblasts to generate cardiomyocytes in the subject; (16) screening (M3) for a candidate substance for an effect on myocardin regulation of cardiomyocyte development, involves providing myocardin and GATA (a cardiac transcription factor) to a cell, admixing myocardin and GATA in the presence of the candidate substance, and measuring the effect of the candidate substance on the expression of a cardiac lineage marker, where a difference in the expression of the cardiac lineage marker, as compared to an untreated cell, indicates that the candidate substance effects myocardin regulation of cardiomyocyte development; (17) screening (M4) for a modulator of myocardin expression, involves providing a cell that expresses a ***myocardin*** ***polypeptide*** , contacting the ***myocardin*** ***polypeptide*** with a candidate substance, and measuring the expression of myocardin, where a difference in myocardin expression, indicates that the candidate substance is a modulator of myocardin expression; (18) screening (M5) a candidate substance for myocardin binding activity, involves providing a ***myocardin*** ***polypeptide*** , contacting ***myocardin*** ***polypeptide*** with the candidate substance, and determining the binding of the candidate substance to the ***myocardin*** ***polypeptide*** ; (19) a method (M6) for modulating the phenotype of a non-cardiomyocyte cell (e.g., fibroblast) to include one or more phenotypic functions of a cardiomyocyte cell, which involves introducing (IV) into the non-cardiac cell, where the promoter directs the expression of the polypeptide; and (20) a method (M7) for generating a cardiomyocyte which involves introducing into a cardiac fibroblast (IV) comprising (I) and a promoter active in the fibroblast, where the promoter directs the expression of the polypeptide.

WIDER DISCLOSURE - The following are disclosed: (1) ***myocardin*** ***polypeptide*** having a sequence of (S1) or

(S2); (2) a polynucleotide sequence encoding (II); (3) sequences that are degenerate with respect to (I); (4) DNA segments that are complementary to (I); (5) variants of ***myocardin*** ***polypeptide*** ; and (6) making (VIII) or (IX).

BIOTECHNOLOGY - Preferred Peptide: (II) preferably comprises 12 consecutive residues of (S1) or (S2). Preferred Polynucleotide: (I) further comprises a promoter operable in eukaryotic cells. (III) is 15, preferably 2000 nucleotides in length, and comprises at least 20, preferably 50 contiguous nucleotides of (S3). Preferred Cassette: In (IV), the regulatory sequence comprises a promoter heterologous to the coding sequence. The promoter is a tissue specific promoter, or muscle specific promoter such as myosin light chain-2 promoter, alpha actin promoter, troponin 1 promoter, Na+/Ca2+ exchanger promoter, dystrophin promoter, creatine kinase promoter, alpha7-integrin promoter, brain natriuretic peptide promoter, alpha B-crystalline/small heat shock protein promoter, alpha myosin heavy chain promoter or atrial natriuretic factor (ANF) promoter. Preferably, (IV) comprises a cardiac muscle specific promoter (e.g., alpha-myosin heavy chain or ANF promoter). (IV) is contained in a gene delivery vector e.g., viral vector such as retroviral vector, adenoviral vector, adeno-associated viral vector, vaccinia viral vector, herpes viral vector, polyoma viral construct or Sindbis viral vector. (IV) further comprises a polyadenylation signal, and a second polynucleotide encoding a second polypeptide e.g., cardiac transcription factor. Preferred Method: The function of myocardin is inhibited by providing to the post-mitotic cardiomyocyte an antisense nucleic acid that inhibits transcription or translation of a myocardin mRNA. Preferably, the method involves introducing into the post-mitotic cardiomyocyte an expression cassette encoding myocardin antisense RNA and a promoter active in the cardiomyocytes. In (M2), the expression cassette is comprised within a replication defective expression vector, e.g., viral vector, and comprises a cardiac specific promoter. In (M3), the effect of the candidate substance on expression of cardiac lineage marker (preferably Nkx2.5) is measured by RNA hybridization, polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), immunologic detection of myocardin, enzyme linked immunosorbent assay (ELISA), immunohistochemistry. The myocardin and GATA are provided to a cell located in an animal e.g., fibroblast, cardiomyocyte. The modulator identified by the method increases or decreases the expression of the cardiac lineage marker. In (M4), the modulator enhances or inhibits myocardin expression. The candidate modulator is a pharmaceutical composition. (M5) is carried out in a cell, cell free system or in vivo. The candidate substance is an inhibitor or enhancer of myocardin. M6 further comprises measuring cardiac lineage markers, by RNA hybridization, polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR) or Western analysis. The expression cassette further comprises a second polynucleotide encoding a cardiac transcription factor (e.g., GATA4). The second polynucleotide is under the control of a second promoter. Optionally, the first and second polynucleotides are under the control of the same promoter. M6 further comprises introducing a second expression cassette into the non-cardiomyocyte cells, where the second expression cassette comprises a polynucleotide encoding a second polypeptide and a second promoter active in the non-cardiomyocyte cells, where the second promoter directs the expression of the second polypeptide. In M7, the expression vector comprises lipid-based vector or a viral vector. Preferred Transgenic Animal: (VIII) is a mouse. (IX) comprises two defective germ-line myocardin alleles.

ACTIVITY - Hypotensive; Cardiant.

MECHANISM OF ACTION - Gene therapy; Antisense therapy; Reprograms cardiac fibroblasts to cardiomyocytes; Inducer of cardiomyocyte development; Inducer of hypertrophy in cardiomyocytes. The effects of myocardin in growth and/or all differentiation of cardiomyocytes was assessed by overexpressing myocardin in cardiomyocytes using adenoviral delivering system. Cardiomyocyte cultures were prepared by dissociation of 1-day-old neonatal rat hearts and were plated differentially to remove fibroblasts. Cells were plated on glass coverslips coated with 4 micrograms/cm2 laminin in 4:1 Dulbecco's modified Eagle's medium (DMEM):199 medium with 10% horse serum and 5% fetal calf serum at a density of 5 x 10 (to the power of 4) cells/cm2. Eighteen hours after plating, cells were changed into serum-free media and infected with adenoviruses expressing either myocardin or beta-galactosidase. For immunofluorescence, cells were fixed in 3.7% formaldehyde on ice for 30 min, permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS) and blocked with 5% serum in PBS for 1 hour at room temperature. Cells were incubated with monoclonal anti-alpha-actinin (sarcomeric) or anti-ANF (atrial natriuretic factor) antibodies at a dilution of 1:200 in blocking buffer for 1 hour at 37 degrees Centigrade, washed and incubated with fluorescein-conjugated horse anti-mouse IgG antibody. Following secondary antibody incubation, cells were washed with PBS. The results showed that overexpression of myocardin in neonatal cardiomyocytes induced assembly of sarcomeres and expression of ANF, markers of cardiac hypertrophy.

USE - (IV) is useful for modulating the phenotype of a non-cardiomyocyte cell (e.g., fibroblast) to include one or more phenotypic functions of a cardiomyocyte cell. (IV) is useful for generating a cardiomyocyte which involves introducing into a cardiac fibroblast (IV) comprising (I) and a promoter active in the fibroblast, where the promoter directs the expression of the polypeptide. (IV) further comprises a second polynucleotide encoding GATA4, under the control of a second promoter active in a cardiac fibroblast. The expression cassette further comprises a polyadenylation site and an immunological marker. (M2) is useful for treating a heart disease, including cardiomyopathy, such as myocardial infarction or hypertension (all claimed).

ADMINISTRATION - Viral vectors comprising (I) are administered by intraarterial or intravenous route in dosages ranging from 1 x 10 (to the power of 4) -1 x 10 (to the power of 12) infectious particles.

EXAMPLE - Expression of myocardin 1 was determined by whole-mount or section in situ hybridizations to mouse embryos at E7.75 and E12.5. The results illustrated the expression pattern of myocardin 1 during early heart development. At E13.5, myocardin expression was evident within smooth muscle cells lining the walls of the esophagus and aortic arch arteries, as well as the pulmonary outflow tract. Expression in these smooth muscle cell types was still apparent, but was diminished, by E15.5. Myocardin expression was also detected in smooth muscle cells within the lung and gut, as well as in head mesenchyme, which may serve as a source of smooth muscle cell precursors. Myocardin was not expressed at expressed in detectable levels in skeletal muscle. The expression of myocardin 1 transcripts in adult mouse tissues was analyzed by Northern blot. The results showed that the transcripts were detected only in the heart. To determine the function of myocardin 1, myocardin 1 expression plasmids were transfected into fibroblasts (COS and HeLa cells) along with expression plasmids for the cardiac transcription factor GATA4. 0.1 micrograms of expression plasmid encoding myocardin 1 along with the luciferase plasmids were mixed with 3 mul of the FuGENE 6 and added to

cells in six-well plates. Cells were harvested 48 hr later and luciferase activity was determined in cell extracts. Cytomegalovirus (CMV)-lacZ which contains the lacZ gene under the control of the constitutive cytomegalovirus promoter was included in all transfections as an internal control. to normalize the variations in transfection efficiency. The results demonstrated that myocardin 1, plus GATA4, transctivates regulatory sequences for the cardiac specific homeobox Nkx2.5, which is the earliest marker for the cardiac lineage in vertebrates. Initial searches of DNA sequence databases with myocardin 1 sequence revealed a number of related sequences. Most of these sequences were short sequences (for e.g. expressed sequence tags (ESTs)) that shared homology to only small regions of myocardin 1. None of the sequences located were identified as encoding proteins having any particular function, much less any function related to cell regulation, particularly cardiac cell regulation. However, using these techniques in combination with the information obtained previously regarding the murine myocardin. Two sequences were identified that shared significant homology with myocardin 1. These appeared to be partial sequences from two additional myocardin genes. cDNA clones for these two related genes, now designated myocardin 2 and myocardin 3 were obtained. A comparison of the three myocardin species identified revealed localized regions of high amino acid homology between the proteins, particularly in the carboxyl-terminal transcription activation domain. By Northern analysis, it was shown that the myocardin 2 was ubiquitous, and that myocardin 3 appeared restricted to heart and liver.(104 pages)

L3 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2002:783402 CAPLUS
DN 139:50069
TI Another hat for myocardin
AU Firulli, Anthony B.
CS Department of Physiology, University of Texas Health Science Center, San Antonio, TX, USA
SO Journal of Molecular and Cellular Cardiology (2002), 34(10), 1293-1296
CODEN: JMCDAY; ISSN: 0022-2828
PB Academic Press
DT Journal; General Review
LA English
AB A review on the identification of myocardin, non-DNA binding cofactor for serum response factor (SRF) and its essential role in mediating the cardiogenic functions of SRF. Myocardin is a 938 amino acid protein that contains a glutamine rich domain and a SAF-A/B, Acinus, PIAS that is present in nuclear scaffolding proteins. SRF is a MADs box-contg. transcription factor which shows a high levels of evolutionary conservation from flies to man. A role for myocardin in smooth muscle gene regulation is also presented.
RE.CNT 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 14:16:44 ON 08 DEC 2004)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 14:16:55 ON 08 DEC 2004

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SEA MYOCARDIN (W) (POLYPEPTIDE OR PROTEIN)
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          2   FILE BIOTECHABS
          2   FILE BIOTECHDS
          2   FILE CAPLUS
         55   FILE DGENE
          1   FILE IFIPAT
          1   FILE USPATFULL
          2   FILE WPIDS
          2   FILE WPINDEX
L1         QUE MYOCARDIN (W) (POLYPEPTIDE OR PROTEIN)
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FILE 'BIOTECHDS, CAPLUS, WPIDS, IFIPAT, USPATFULL' ENTERED AT 14:18:32 ON 08 DEC 2004

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L2            8 S L1
L3            4 DUP REM L2 (4 DUPLICATES REMOVED)
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L2	3	myocardin polypeptide	US-PGPUB; USPAT; DERWENT	ADJ	ON	2004/12/08 14:41
S1	1	"6569662".pn.	USPAT	ADJ	ON	2004/12/08 14:38